

preparative regimen consisting of busulfan, cyclophosphamide, and anti-thymocyte globulin for a 5 out of 6 HLA-antigen-matched unrelated donor cord blood transplant. Graft-versus-host disease (GVHD) prophylaxis consisted of cyclosporine and methylprednisolone, and the patient experienced isolated acute skin GVHD. Notably, several astrocytoma lesions regressed or decreased in size between 3 and 8 months after transplantation, associated with a lymphocytic CSF pleiocytosis with elevated CSF protein. The patient remains leukemia-free with stable brain lesions 25 months after transplantation. This case illustrates the potential for donor-derived immune cells to control brain tumors, and suggests that allogeneic bone marrow transplantation may represent an important immunotherapeutic strategy for the aggressive treatment of poor-prognosis brain tumors. Future clinical protocols should seek to further characterize the nature of the immune response to brain tumors after allogeneic bone marrow transplantation.

STEM CELL BIOLOGY

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USE OF CHROMATIN MODIFYING AGENTS FOR EX VIVO EXPANSION OF HUMAN UMBILICAL CORD BLOOD STEM CELLS

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The fixed number of hematopoietic stem cells (HSCs) within a single cord blood (CB) unit has limited the use of CB for allogeneic transplantation in adults. Efforts to promote self-renewal and expansion of HSCs have been met with limited success. Using presently available ex vivo culture techniques, HSCs lose their functional properties in proportion to the number of cellular divisions they have undergone. We hypothesized that chromatin modifying agents, 5-aza-2'-deoxycytidine (5azaD) and histone deacetylase inhibitor, trichostatin A (TSA) could reactivate pivotal genes required for retaining the functional properties of dividing HSC to permit transplantation of adults. A 12.5-fold expansion was observed in the 5azaD/TSA treated CD34+CD90+ cell cultures containing SCF, thrombopoietin, and FLT3 ligand (cytokines) in comparison to the input cell number. Despite 9 days of culture, $35.4\% \pm 5.8\%$ ($n = 10$) of the total cells in the cultures exposed to chromatin modifying agents were CD34+ CD90+ compared to $1.40\% \pm 0.32\%$ in the culture containing cytokines alone. The CD34+CD90+ cells were associated with a 9.8-fold increase in the numbers of CFU-mix and 11.5-fold expansion of cobblestone area-forming cells (CAFC). The frequency of SCID repopulating cells (SRC) was 1 in 26,537 in primary CB CD34+CD90+ cells but was increased to 1 in 2745 CD34+CD90+ cells following 9 days of culture in the presence of 5azaD/TSA resulting in a 9.6-fold expansion of the number of SRC. In contrast, the cultures lacking 5azaD/TSA had a net loss of both CFC/CAFC as well as SRC. The expansion of cells maintaining CD34+CD90+ phenotype was not due to the retention of a quiescent population of cells since all of the CD34+CD90+ cells in the culture had undergone cellular division. CD34+CD90+ cells that had undergone 5–10 cellular divisions in the presence of 5azaD/TSA but not in the absence still retained the ability to repopulate NOD/SCID mice. We next assessed the effect of 5azaD/TSA treatment on the expression of *HOX-B4*, a transcription factor which has been implicated in HSC self-renewal. A significantly higher level of *HOXB4* protein was detected by western blot analysis after 9 days of culture in the cells treated with 5azaD/TSA as compared to cells exposed to cytokines alone. The almost 10-fold increase in SRC achieved using the chromatin modifying agents may be sufficient to increase the numbers of engraftable HSC within a single human CB unit for adult recipients.

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ADULT HUMAN HEMATOPOIETIC CELLS DIFFERENTIATE INTO MATURE T CELLS VIA A CD3-4+8- INTERMEDIATE WITHIN THE MOUSE THYMIC MICROENVIRONMENT; A NEW MODEL SYSTEM FOR THE STUDY OF HUMAN THYMOCYTE DEVELOPMENT FURTHER ENHANCED BY ANTI-MURINE c-Kit mAb

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Normal T cell differentiation occurs within the thymus. We previously found that human thymocyte precursors develop via a novel CD3-4+8- intermediate population, utilizing SCID-hu thymic grafts (Kraft, Weissman, Waller; JEM). We have recently worked to develop a more convenient robust model of human hematopoiesis utilizing RAG2/Common Gamma Chain double KO mice transplanted with hematopoietic progenitor cells from adult human donors, and hypothesized that human engraftment would be enhanced by selective inhibition of murine hematopoiesis by administration of anti-murine c-Kit mAb. **Methods:** Mobilized CD34+ cells from healthy adults were obtained to >90% purity by CliniMacs. $2-8 \times 10^5$ CD34+ cells were injected intrahepatically into newborn RAG2 DKO pups following 4Gy of irradiation. At serial time points, human CD45+ chimerism was measured within the recipient marrow, blood, spleen, liver, lymph node, and thymus. A subset of recipients were treated with the anti-murine c-Kit mAb (ACK2) starting at D +14. **Results:** Human engraftment was detectable in CD34+ transplanted mice, and this was enhanced by suppression of murine hematopoiesis by anti c-Kit mAb. Robust human thymopoiesis was observed. A mean of 63% of cells within the thymus were human derived. At earlier time points (4–6 weeks post transplant) the recipient thymus were found to contain high fractions of CD45+CD3-4-8- cells (making up 30–40% of human cells within the thymus) and the CD3-4+8- (20–30%) and CD4+8+ (30–70%) intermediate populations with very rare mature CD3+4+8- or CD3+4-8+ T cells. At later time points the fraction of immature CD3-4-8- and CD3-4+8- populations declined and increasing populations of mature CD3+4+8- and CD3+4-8+ populations were identified in distributions similar to a normal thymus. **Conclusions:** Human T cell development appears to progress normally within a murine thymic microenvironment. The early development of a CD3-4+8- intermediate suggests that T cell development occurs via this population, unlike the CD3-4-8+ intermediate found in mice thymopoiesis, suggesting that the pathway of human T cell differentiation is intrinsic to the human thymocytes, and is independent of whether the thymic stroma is human or murine. This robust model system enabling study of human thymopoiesis utilizing hematopoietic stem cells from normal and diseased adults human donors may provide significant advantages for the study of human intrathymic T cell differentiation and function in vivo.

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CD8⁺/TCR⁻ GRAFT FACILITATING CELLS ENHANCE HSC FUNCTION VIA INDUCTION OF LOW LEVELS OF TNF- α

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Approaches to enhance engraftment of HSC when cell numbers are limiting remain an elusive goal in bone marrow transplantation. We recently reported that CD8⁺/TCR⁻ facilitating cells (FC), a subpopulation of BM cells containing predominantly B220⁺/CD11c⁺/CD11b⁻ tolerogenic precursor-plasmacytoid dendritic cells, enhance HSC engraftment in allogeneic recipients. FC are themselves tolerogenic, directly preventing GVHD. Additionally, FC significantly enhance engraftment of limiting numbers of HSC in syngeneic recipients. In the present studies, we investigated the mechanism of FC function. Here we show for the first time that FC significantly increase HSC